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Short report

Vanadium accelerates polymerase chain reaction and expands the applicability of forensic DNA testing

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ABSTRACT

Polymerase chain reaction (PCR) has been rapidly established as one of the most widely used techniques in molecular biology. Because most DNA analysis is PCR-based, the analysis of unamplifiable DNA of poor quality or low quantity is nearly impossible. However, we observed that if an appropriate concentration of vanadium chloride is added to the standard reaction mixture, the enzymatic amplification of DNA could be enhanced. Using multiplex PCR with the addition of vanadium, DNA typing was possible from even trace amounts of DNA that we were unable to amplify using normal reaction conditions.

This method might be an effective tool for not only criminal investigations and ancient DNA analysis, but also for nearly all fields using DNA technology.

1. Introduction

Often in forensic DNA testing, we are unable to amplify sample DNA of low quantity and quality by polymerase chain reaction (PCR), which makes DNA testing impossible.

PCR is now routinely used in almost all molecular biology research.[1] PCR is an *in vitro* method for the million-fold amplification of specific DNA sequences by the simultaneous primer extension of the complementary strands of the target DNA.[2]

After the discovery of the heat-stable DNA polymerase (Taq polymerase) isolated from *Thermus aquaticus* and the standard buffer formulation established by Saiki et al.,[3,4] it became possible for PCR to be performed by an automated system using a thermal cycling device. However, there are problems with PCR sensitivity, especially when dealing with poor quality, degenerate or low-copy-number DNA templates in forensic science[5,6] and evolutionary biology. In forensic identification, material evidence obtained from a crime scene is often severely limited, which poses a problem for detection by PCR.

Increasing the sensitivity and specificity of PCR are major goals and would be very valuable for fields using PCR. Hence, we investigated whether vanadium, one of the metallic elements in the crust of volcanic rocks, has remarkable abilities to enhance PCR for application to forensic DNA analysis.

2. Materials and methods

2.1. PCR conditions

We used commercially available genomic human DNA (AmpFlSTR DNA Control 007, Applied Biosystems, Carlsbad, CA, USA) as the PCR template. Amplification was done using the Y-STR loci, DYS19,[7,8] and a control mitochondrial DNA (mtDNA) region (L16200-H127) because these loci could be amplified as a single band so that we could easily evaluate band intensity.

PCR amplification was performed in a GeneAmp PCR System 9600 instrument (Applied Biosystems, Carlsbad, CA, USA) with 1 × PCR Buffer II, 0.2 mM deoxynucleotide triphosphate (dNTPs), 1.5 mM $MgCl_2$, 4% glycerol, 0.2 μM each primer, 1 unit of HotStarTaq polymerase (Qiagen, Hiden, Germany) and 50 pg genomic DNA in a total volume of 12 μl.

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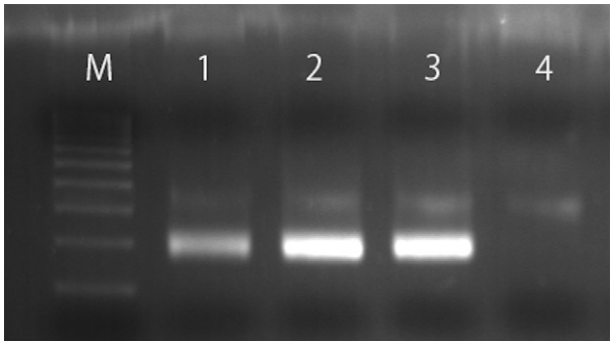

Fig. 1. The effect of appropriate concentration of VCl_4 into PCR. Human genomic DNA templates of 50 pg in 12 μl standard reaction buffer were used. The human Y chromosomal microsatellite DYS19 locus was used with various concentrations of VCl_4 (ALDRICH), ranging from 0–1.7 μg/ml. M: 100-bp Molecular Ruler (BIO-RAD). 1: VCl_4(0.5 μg/ml), 2: VCl_4(1.5 μg/ml) 3: VCl_4(1.7 μg/ml), 4: VCl_4 (0 μg/ml).

For the PCR thermocycle, we set the three steps as follows: After initial denaturation at 94 °C (10 min), a three-step cycle of 94 °C for 1 min (denaturation), 57 °C for 1 min (annealing) and 72 °C for 1 min (extension) was repeated 28 times.

In order to evaluate the assay sensitivity, appropriate concentrations of vanadium tetrachloride (VCl_4) were added to the buffer in a dilution series from 0 to 2.4 μg/ml.

Exact vanadium concentrations were measured using an inductively coupled plasma (ICP) Emission Spectrochemical Analyser (SII NanoTechnology Inc., Chiba, Japan).

2.2. Real-time PCR assays

The effect of VCl_4 to enhance the PCR reaction was quantified using the SYBR Green Master Mix™ Kit (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer's protocol on an Real-Time PCR system 7500 (Applied Biosystems, Carlsbad, CA, USA). Vanadium tetrachloride (VCl_4) was added to the buffer in a dilution series of 0, 0.5, 0.7, 1.5 and 1.7 μg/ml final concentrations.

2.3. Application to the multiplex PCR kit

We performed an experiment to examine the effectiveness of the addition of vanadium to the personal identification kits that are widely used in forensic DNA testing.

We tested the addition of vanadium tetrachloride (VCl_4) with the AmpFLSTR™ Yfiler and AmpFLSTR™ Identifiler kits (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer's instructions, using Control DNA 007 and AmpliTaq Gold (Applied Biosystems, Carlsbad, CA, USA).

PCR product sizing was performed by capillary electrophoresis with an ABI PRISM™3130xl genetic analyser (Applied Biosystems, Carlsbad, CA, USA) and genotyping was carried out with Gene Mapper ID™ v3.2 (Applied Biosystems, Carlsbad, CA, USA). The genotypes of all samples were determined and compared to the results obtained from each control allelic standard sample.

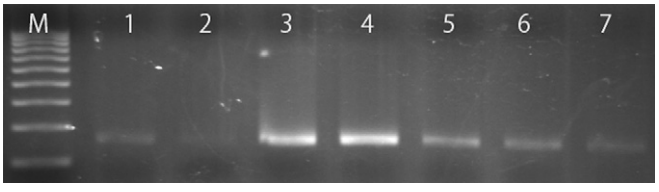

Fig. 2. Amplifications of DYS390. Template DNA (control DNA 007) was prepared to 50 pg. Vanadium tetrachloride (VCl_4) was added in the buffer in a dilution series: M: 100-bp Molecular Ruler (BIO-RAD). 1: VCl_4 (1.0 μg/ml): 2: VCl_4 (1.2 μg/ml), 3: VCl_4 (1.5 μg/ml), 4: VCl_4 (1.7 μg/ml), 5: VCl_4 (2.0 μg/ml). 6: VCl_4 (2.2 μg/ml), 7: VCl_4 (2.4 μg/ml).

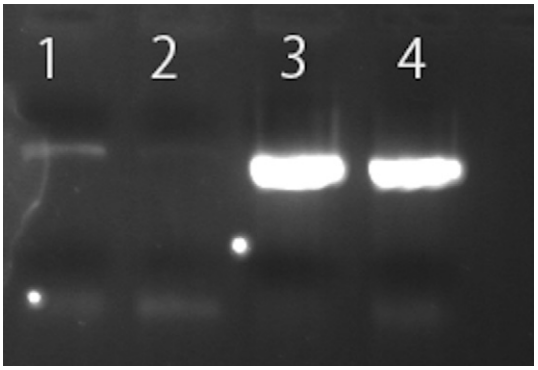

Fig. 3. Amplifications of mtDNA. VCl_4 was added in the buffer in a dilution series: 1: VCl_4 (0 μg/ml), 2: VCl_4 (0.2 μg/ml), 3: VCl_4 (1.5 μg/ml), 4: VCl_4 (1.7 μg/ml). A maximal amplification effect was obtained with the addition of 1.5–1.7 μg/ml VCl_4.

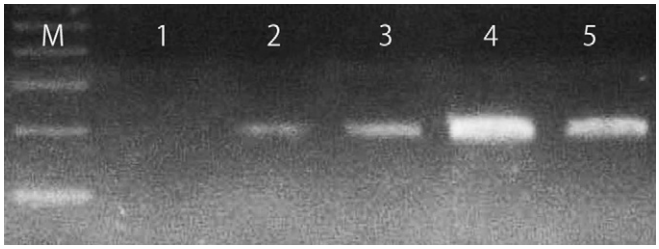

Fig. 4. Amplifications of DYS19. Template DNA (control DNA 007) was added in the buffer (including 1.5 μg/ml of VCl_4) in a dilution series (20 pg–1000 pg): M: 100-bp Molecular Ruler (BIO-RAD). 1: Template 20 pg, 2: template 50 pg, 3: template 100 pg, 4: template 200 pg, 5: template 500 pg. Amplification band was visible using as low as 50 pg template.

3. Results

We found that vanadium enhances PCR remarkably, as shown in Fig. 1. The enhancement effect is found at a wide range of

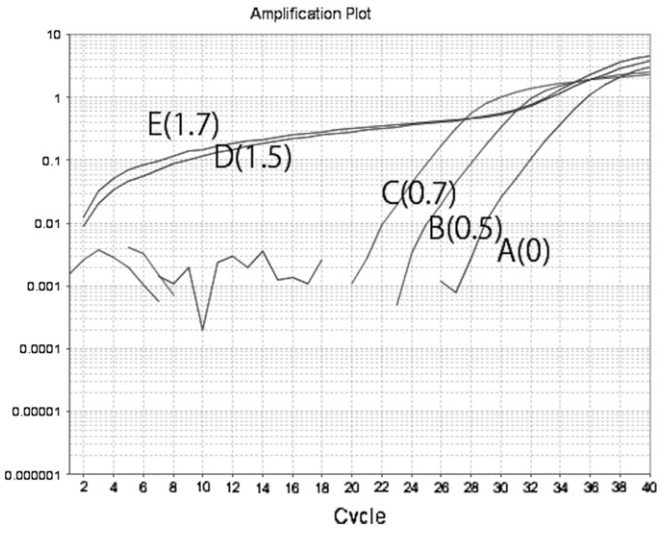

Fig. 5. Amplification plots of real-time PCR assay using SYBR Green Master Mix Kit (Applied Biosystems). Target locus was mtDNA. Control DNA 007 was prepared to 100 pg. Vanadium tetrachloride (VCl_4) was added in buffer, with a dilution series including A: VCl_4 (0 μg/ml), B: VCl_4 (0.5 μg/ml), C: VCl_4 (0.7 μg/ml) D: VCl_4 (1.5 μg/ml) E: VCl_4 (1.7 μg/ml). A maximal amplification effect was obtained with the addition of 1.5–1.7 μg/ml VCl_4.

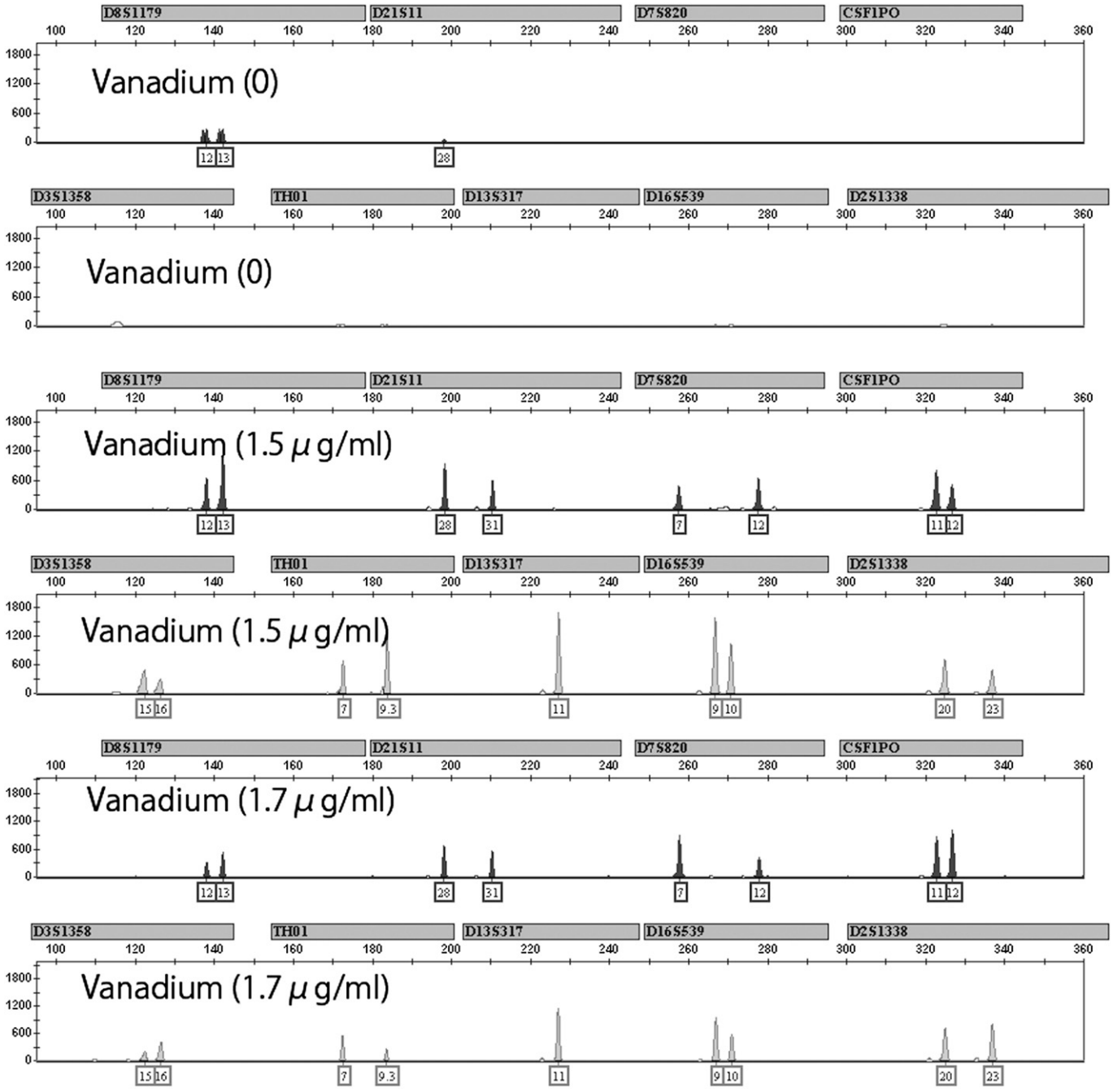

Fig. 6. Electropherograms of the DNA typing with the Identifiler™ kit using 50 pg template DNA (control DNA 007). Ist–2nd Column: no vanadium, 3rd–4th: column: VCl_4 (1.5 μg/ml), 5th–6th: column: VCl_4 (1.7 μg/ml). The longitudinal maximal scale was set to 2000 RFUs.

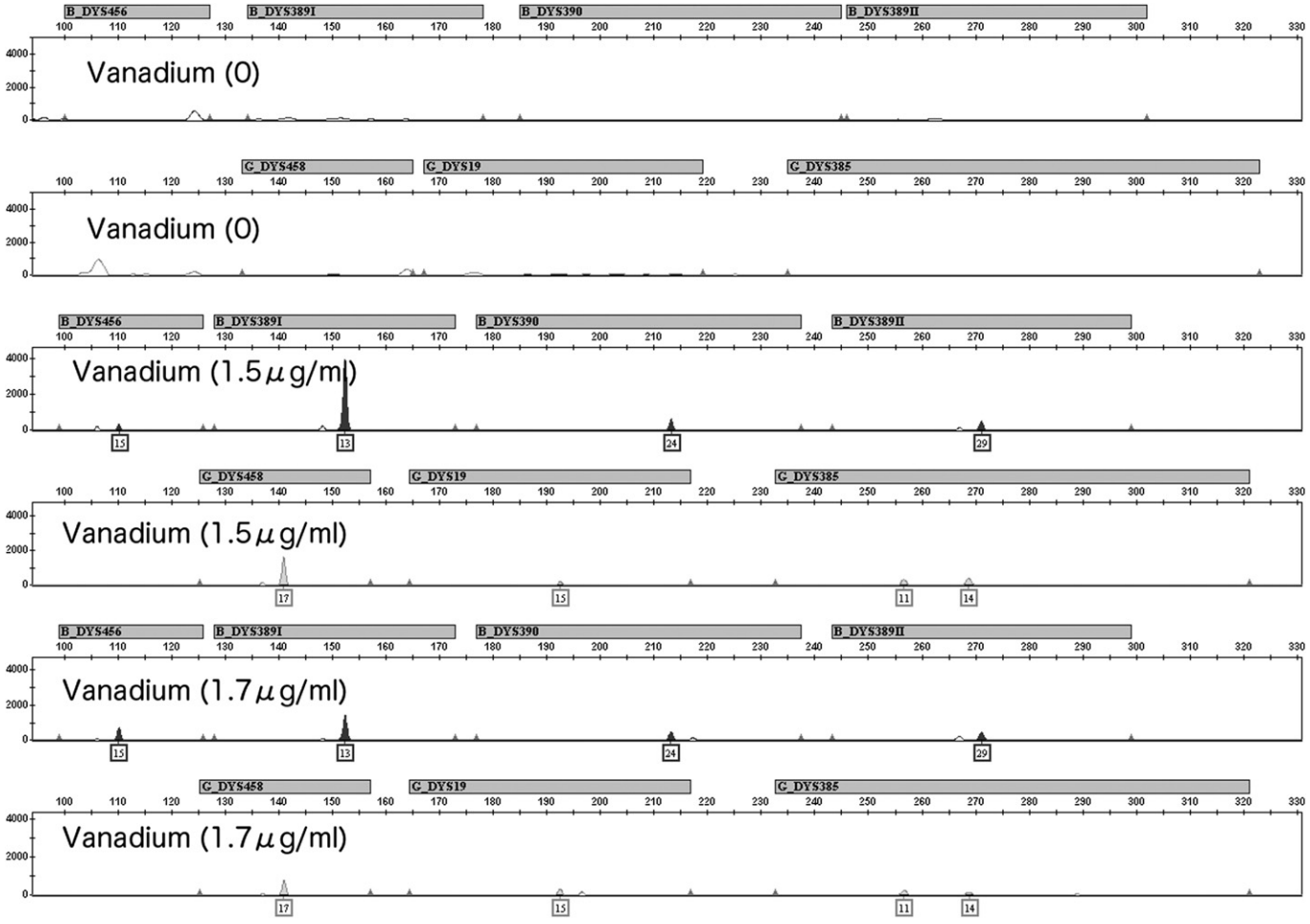

Fig. 7. Electropherograms of the DNA typing using the Yfiler™ kit with 50 pg template DNA (control DNA 007). Ist–2nd Column: no vanadium, 3rd–4th column: VCl_4 (1.5 μg/ml), 5th–6th column: VCl_4 (1.7 μg/ml). The longitudinal maximal scale was set to 5000 RFUs.

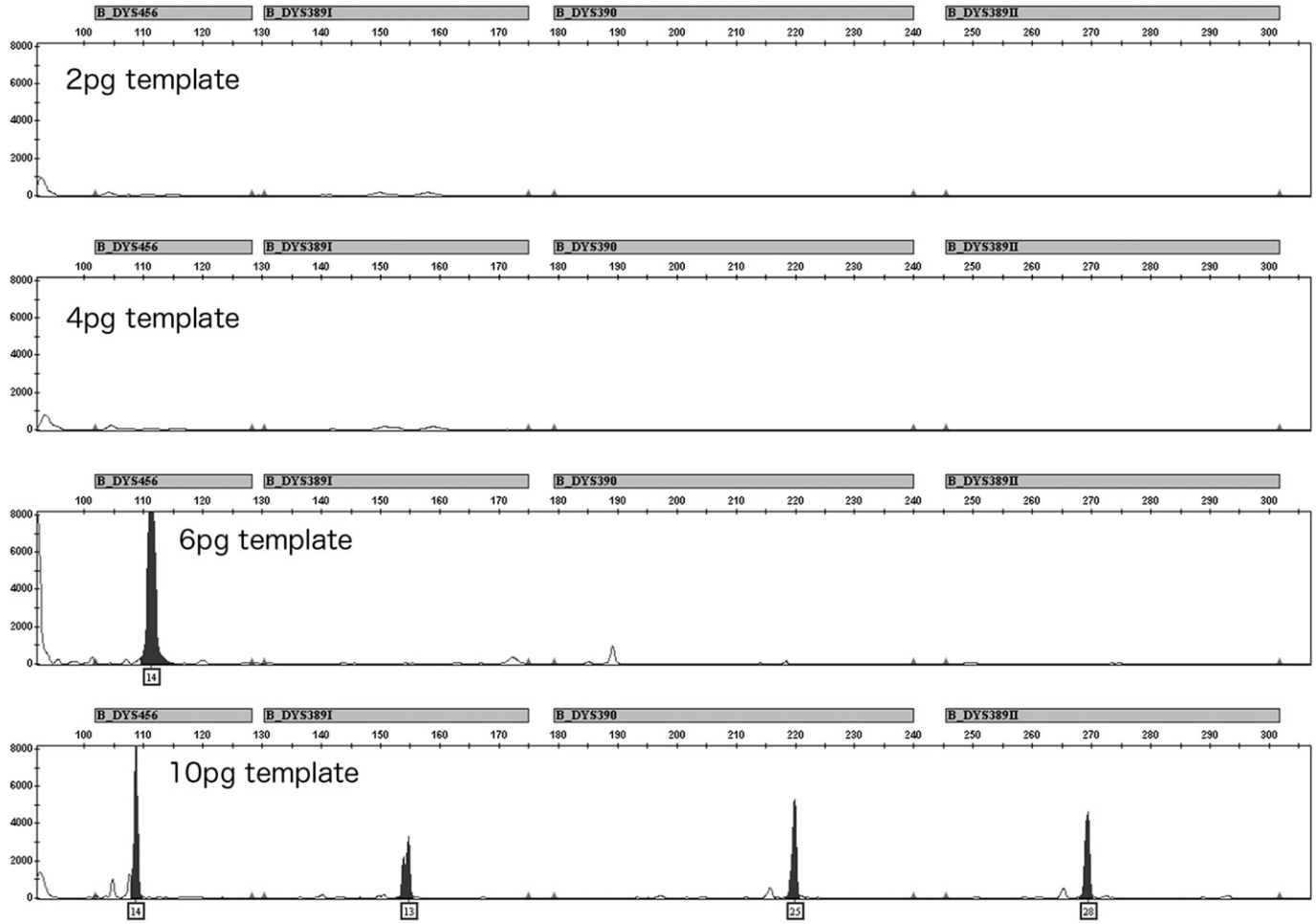

Fig. 8. Electropherograms of the DNA typing with the Yfiler™ kit using 2–10 pg male template DNA with 1.5 μl/ml vanadium. Ist Column: DNA typing from 2 pg template DNA, 2nd column: DNA typing from 4 pg template DNA, 3rd column: DNA typing from 6 pg template DNA, 4th column: DNA typing from 10 pg template DNA. The longitudinal maximal scale was set to 8000 RFUs. For full profiling, we can detect from as low as 10 pg template DNA.

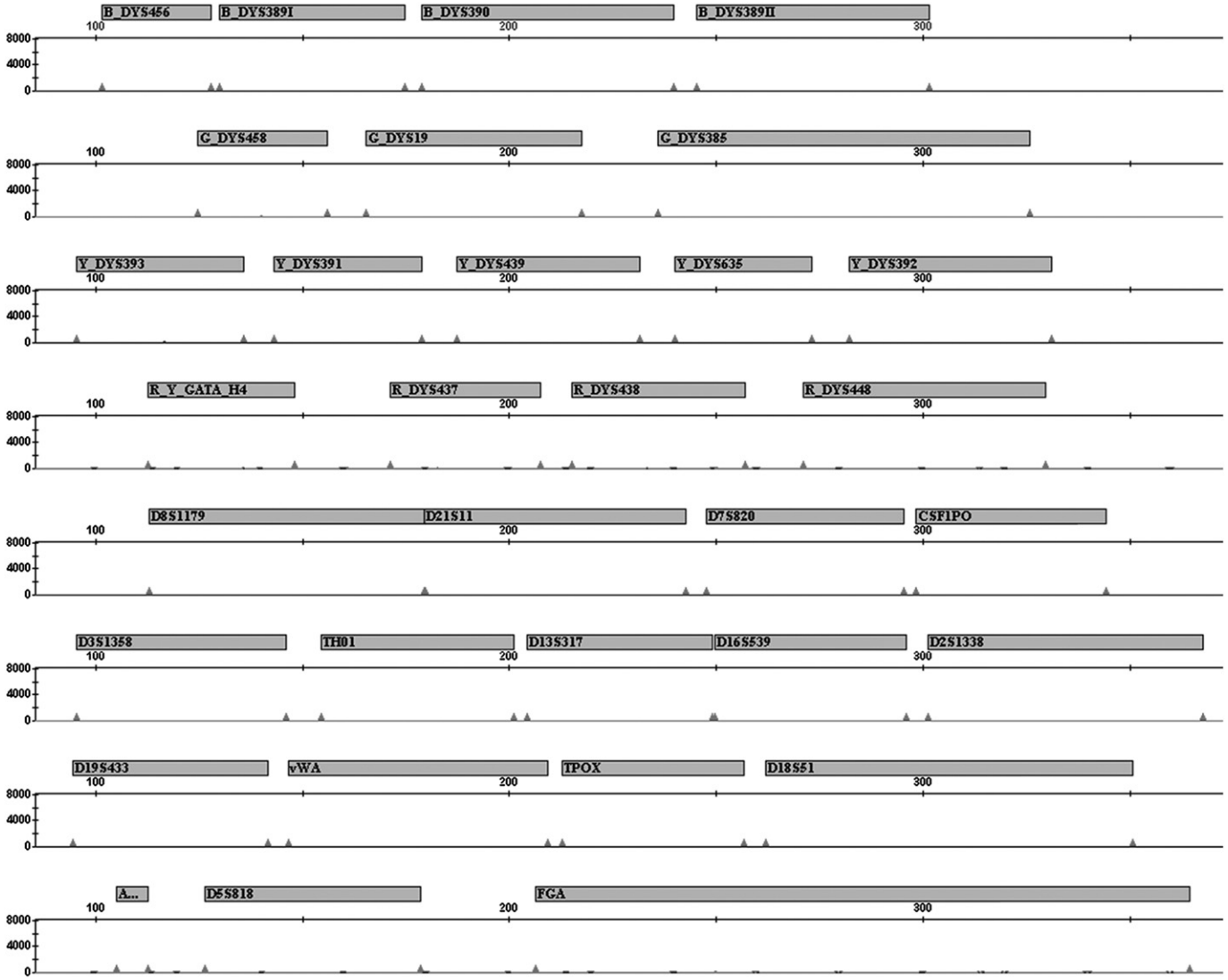

Fig. 9. Electropherograms of the DNA typing with the Identifiler and Y-filer™ kit with no vanadium using 500 pg template DNA from victim's bone which passed after death for three years (no vanadium). Amplifications were failed.

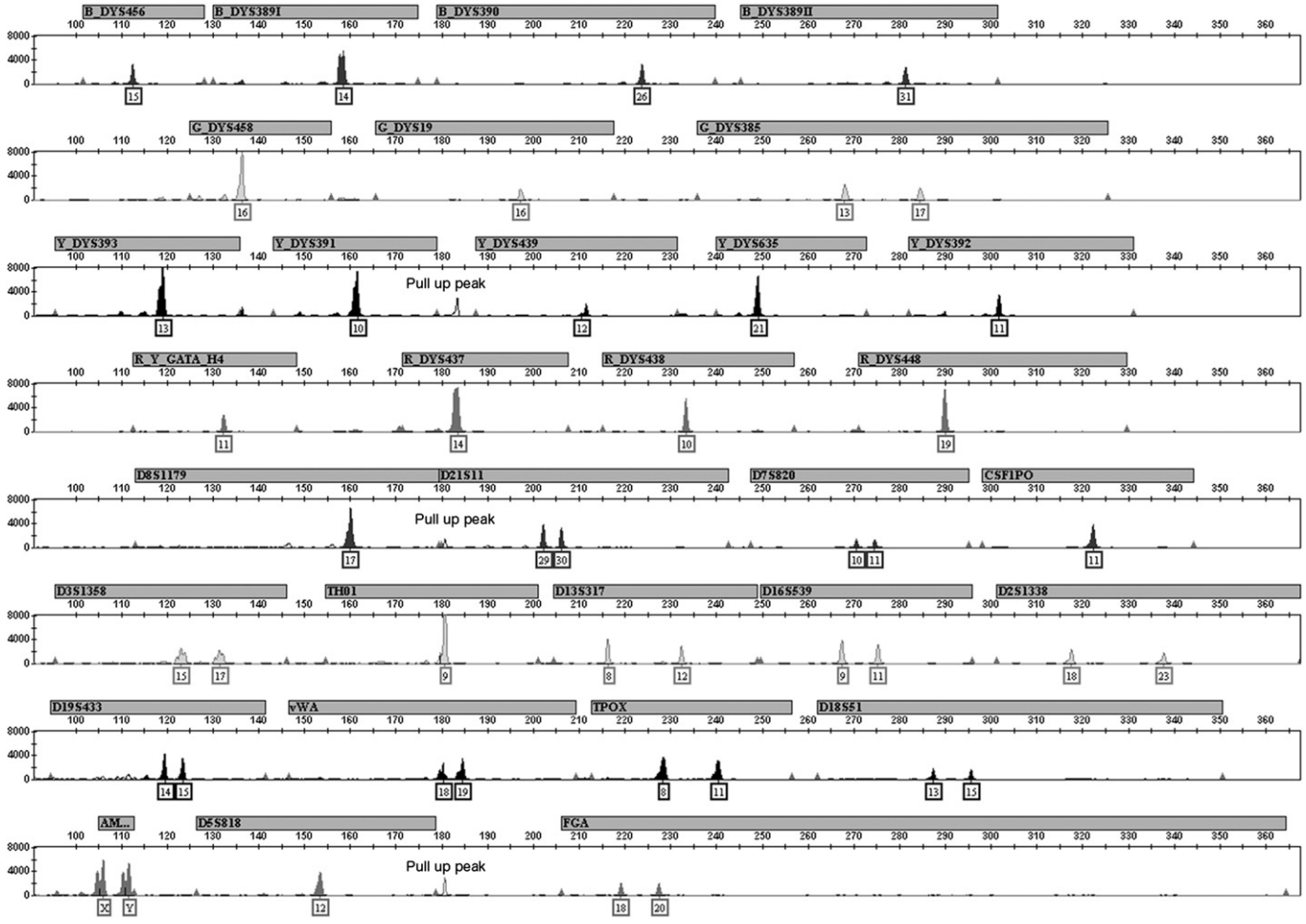

Fig. 10. Electropherograms of the DNA typing with the Identifiler and Y-filer™ kit using 1.5 μg/ml vanadium, and 500 pg template DNA from victim's bone which passed after death for three years. We can get full profiling data.

concentrations, from 0.5 to 1.7 μg/ml (VCl_4) in the reaction mixture. The most effective concentration of VCl_4 is 1.5–1.7 μg/ml.

Similarly, a maximum amplification effect was obtained with the addition of 1.5–1.7 μg/ml VCl_4 (Figs. 2 and 3).

In addition, the lowest amplifiable template amount in the PCR using VCl_4 was visible to be 50 pg (Fig. 4). Explosive amplifications were already confirmed in an early cycle stage by the real-time PCR assay using 1.5–1.7 μg/ml (VCl_4). The assay also suggested that 1.5–1.7 μg/ml VCl_4 was optimal (Fig. 5).

In addition, because the enhancement effect was found to be worked in the same way whether we used HotStarTaq™ or AmpliTaq™ Gold, vanadium did not have efficacy in response to a special enzyme. In addition, the effects of the vanadium worked in the same way by PCR of autosomal STR, Y STR and mtDNA.

When vanadium was added to the reaction buffer of any multiplex kit to the optimal concentration, the nonspecific band (noisy background, or Stutter peak, etc.) did not appear. These results suggest that vanadium did not induce a nonspecific PCR reaction (Figs. 6 and 7).

By testing of a range of DNA template concentrations, the new technologies are developed that allow very low quantities of DNA, as low as a 10 pg template, to be detected (Fig. 8).

Much DNA extracted from samples collected from real crime scenes; DNA extracted from old bones of victims can be tested by this method (Figs. 9 and 10). Although the standard method with no vanadium failed, vanadium addition enables amplification of PCR so that all samples can be amplified and typed by this method.

4. Discussion

The relationship between vanadium and DNA amplification was previously unknown. However, we suspected that vanadium strongly induced annealing reactions from template DNA directly, because we found explosive amplification in the early stage of PCR by real-time PCR assay (Fig. 5).

In the experiment by multiplex PCR using vanadium addition, DNA typing was possible even from trace amounts of the DNA that could not be amplified under the normal reactive conditions.

Vanadium, which was first discovered in Sweden in 1831 by Sefström in iron ore, is a metallic element (atomic number 23, molecular weight 50.9) that is widely distributed in the crust of volcanic rocks and concentrated in petroleum.[9] From these observations, one might speculate that DNA activity is strongly associated with volcanic substances. This notion is supported by the existence of the Taq polymerase, which was originally isolated from a hot spring and first described 43 years ago.[10] Vanadium is known to be an essential trace element for growth and reproduction in mammals.[11] In humans, vanadium is mainly used in medicine because it was discovered to have an insulin-like pharmacological effect to normalise blood glucose levels.[12,13] In addition, cells exposed to vanadium were found to activate a nuclear factor of activated T cells,[14] suggesting a relationship between vanadium and DNA.

The method presented here is not only effective for criminal investigations and DNA diagnostics in medicine but for research on DNA derived from extinct animals and plants in archaeology.

5. Conclusion

This study provides the first evidence that PCR is remarkably enhanced by the addition of vanadium. The optimal concentration of vanadium in the PCR reaction is approximately 1.5–1.7 μg/ml. At this vanadium concentration, an effect is obtained from as low as 10 pg of template DNA for complete profiling by a multiplex DNA typing kit.

Supplementary

The method presented here has been patented (#4224522) by the Japanese Patent Office.

When any company wants to commercialize the method that we showed by this paper, please consult with the corresponding author.

Ethical approval

This work described in our manuscript have been, and will be carried out in accordance with The Code of Ethics of the World Medical Association for experiments involving humans.

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All authors have not any financial and personal relationship with other people or organisations concerning our work.

Author contribution

Our work presented here, has not been published previously, and not under consideration for publication elsewhere, and submission and publication of this manuscript are approved by all authors.

Conflict of interest

None declared.

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